Patent
260/243

AMENDMENTS

IN THE CLAIMS:

Please cancel claims 1-52.

Please add the following claims:

53 ~~1~~. A method for purifying plasmid DNA, the method comprising the steps of:

(a) lysing cells containing the plasmid DNA, wherein said lysing step comprises:

moving a suspension of the cells through a first passageway;

moving a lysis solution through a second passageway;

contacting the suspension of the cells with the lysis solution at an intersection, wherein the intersection is formed by the first and second passageway; and

mixing the suspension of the cells and the lysis solution inside a third passageway to form a lysate, wherein the third passageway is downstream from the intersection;

(b) removing contaminants from the lysate, wherein the removing step comprises:

moving the lysate through the third passageway and into a container, wherein the container contains a salt solution; and

mixing the lysate in the salt solution to form a precipitate and a supernatant; wherein the precipitate contains the contaminants;

(c) separating the precipitate from the supernatant; and

(d) recovering the plasmid DNA from the supernatant.

2. The method in claim 1 wherein the third passageway comprises an in-line mixer.

3. The method in claim 1 wherein the container has an impeller mixer for mixing the lysate in the salt solution.

4. The method in claim 1 wherein the container is a holding tank having a jacket.

5. The method of claim 1 wherein the container has the capacity to contain a volume of about 100 to about 200 liters.

6. The method of claim 1 wherein the recovering of plasmid DNA yields at least about 60%.

7. The method of claim 1 wherein the plasmid DNA is a supercoiled plasmid DNA.

8. The method of claim 7 wherein the supercoiled plasmid DNA is enriched above 80%.

9. The method in claim 1 further comprising a cell resuspension step before the lysing step, wherein the resuspension step comprises:

recirculating the cells and a resuspension solution through a fourth passageway; wherein the cells and the resuspension solution are mixed inside the fourth passageway.

10. The method in claim 9 wherein the fourth passageway comprises an in-line mixer.

11. The method in claim 1 wherein the step of removing the contaminants further comprises chilling the precipitate and supernatant in the container at 4 degree Celsius for a time between about 6 to about 12 hours, and wherein the chilling step is before the step of separating the precipitate from the supernatant.

12. The method in claim 1 wherein the suspension of cells, the lysis solution, and the lysate are moved at a flow rate of about 1 liter/min.

13. The method in claim 1 wherein the salt solution is a non-acidic salt solution.

14. The method in claim 1 wherein the salt solution is unbuffered salt solution.

15. The method in claim 1 wherein the salt solution has a salt concentration of greater than 5M.

16. The method of claim 13 wherein the non-acidic salt solution comprises a salt selected from a group consisting of acetate salt and chloride salt.

17. The method of claim 16 wherein the acetate salt has a concentration of about 8M.

18. The method of claim 17 wherein the acetate salt is a mixture of potassium acetate and ammonium acetate.

19. The method of claim 18 wherein potassium acetate is at a concentration of about 1M and ammonium acetate is at a concentration of about 7M.

20. The method of claim 13 wherein the non-acidic salt solution has a pH between 7 to 9.

21. The method in claim 13 wherein the non-acidic salt solution comprises a mixture of two salts, the salts being selected from a group consisting of potassium acetate, potassium chloride, sodium chloride, and ammonium acetate.

22. The method of claim 1 wherein the lysis solution is basic.

23. The method of claim 22 wherein the lysis solution has a pH of about 12-13.

24. The method of claim 23 wherein the lysis solution comprises 0.2N sodium hydroxide and a detergent selected from a group consisting of SDS, triton X, Tween, sarkosyl, and NP-40.

25. The method in claim 1 wherein the precipitate comprises contaminants selected from the group consisting of RNA, chromosomal DNA, lipids, and protein.

26. The method in claim 1 wherein the step of recovering the plasmid DNA from the supernatant comprises exposing the supernatant to column chromatography.

27. The method in claim 26 wherein the step of exposing the supernatant to column chromatography involves the use of a hydrophobic interaction column.

28. The method in claim 27 wherein the hydrophobic interaction column comprises a resin selected from a group consisting of: Octyl Sepharose 4 Fast Flow, Phenyl Sepharose, Butyl Sepharose, Phenyl 650-S.

29. The method in claim 26 wherein the step of exposing the supernatant to column chromatography comprises:

adjusting a salt concentration of the supernatant to have at least 2M ammonium sulfate;

flowing the adjusted supernatant through an hydrophobic interaction column; and

eluting the plasmid DNA with an eluent solution having less than 2M ammonium sulfate.

30. The method in claim 29 wherein the eluent solution has ammonium sulfate at a concentration of less than or equal to 1.6M.

31. The method in claim 26 wherein the step of exposing the supernatant to column chromatography comprises:

adjusting a salt concentration of the supernatant to have about 1.6M to about 1.75M ammonium sulfate;

flowing the adjusted supernatant through an hydrophobic interacting column; and

collecting a flow through eluate flowing from the hydrophobic interacting column.

32. The method in claim 27 wherein the step of exposing the supernatant to column chromatography further involves the use of an anionic exchange column.

33. The method in claim 32 wherein the use of an anionic exchange column comprises eluting plasmid DNA via a step gradient.

34. The method in claim 32 wherein the anionic exchange column comprises a resin having a particle size of 20-40 microns.

35. The method in claim 32 wherein the anionic exchange column comprises a resin selected from a group consisting of: Fractogel EMD TMAE (650-S), Fractogel (R) EMD TMAE Hicap, Q Sepharose 4 Fast Flow, DEAE 650-S

36. The method in claim 32 wherein the plasmid DNA is eluted from the anionic exchange column with a salt solution selected from a group consisting of: about 1.9 M ammonium sulfate and at least 0.7M NaCl.

37. The method in claim 26 wherein the step of exposing the supernatant to column chromatography further comprises the steps of:

passing the supernatant through the anionic exchange column; wherein the plasmid DNA binds to the anionic exchange column;

eluting the plasmid DNA in a first eluate from the anionic exchange column;

adjusting a salt concentration of the first eluate to contain at least 2M ammonium sulfate;

passing the first eluate through the hydrophobic interaction column; wherein supercoiled plasmid DNA binds to the hydrophobic interaction column; and

eluting the supercoiled plasmid DNA in a second eluate from the hydrophobic interaction column via an eluent having less than 2M ammonium sulfate.

38. The method of claim 37 wherein the plasmid DNA in the first eluate is eluted from the anionic exchange column with a salt solution selected from a group consisting of: about 1.9 M ammonium sulfate and at least 0.7M NaCl.

39. A device for purifying plasmid DNA from cells containing said plasmid DNA, the device comprising:

a first in-line mixer for resuspending the cells into a homogenous cell suspension;

a second in-line mixer in fluid connection with the first in-line mixer for mixing the homogenous cell suspension with a lysis solution to form a lysate;

a container for holding a salt solution, wherein the holding tank comprises:

a mixer for mixing a salt solution in the holding tank with the lysate flowing from the second in-line mixer to form a precipitate and a supernatant; and

a container outlet from which the precipitate and supernatant are removed from the tank; and

at least one pump for causing the cells to flow through the first in-line mixer and the homogeneous cell suspension and lysis solution to flow through the second in-line mixer.

40. The device in claim 39 wherein the container has a capacity to hold a volume of about 100 to about 200 liters.

41. The device in claim 39 further comprising a chiller that chills the container to at least 10 degree Celsius or below.

The device in claim 41 wherein the container holding tank having a jacket and wherein the chiller comprises a recirculating chiller connected to the jacket.

42. The device in claim 39 further comprising:

a reservoir for holding a resuspension solution and the cells, and wherein the reservoir is in fluid connection with the first in-line mixer.

43. The device in claim 42 wherein the first in-line mixer comprises a tubing having :

a tubing inlet connected to the reservoir, said tubing inlet allowing the resuspension solution and the cells to flow into the tubing from the reservoir; and

a tubing outlet directed back at the reservoir such that an effluent from the tubing flows back into the reservoir.

44. The device in claim 39 wherein the second in-line mixer comprises:

a first inlet whereby the homogenous cell suspension enters the second in-line mixer through the first inlet;

a second inlet whereby the lysis solution enters the second in-line mixer through the second inlet; and

wherein the first and second inlet joins at an intersection to form an outlet.

45. The device of claim 44 wherein the intersection is a Y-shaped intersection.

46. The device in claim 44 wherein a static mixer is positioned downstream of the outlet.

The device in claim 44 wherein the outlet is connected to a tubing that coils around the container.

47. The device in claim 39 wherein container further comprises a filter connected to the container outlet for filtering the precipitate.

48. The device in claim 47 wherein the holding tank is in fluid connection with a column chromatography system.

49. A device for purifying plasmid DNA from cells containing said plasmid DNA; the device comprising:

a first in-line mixer for resuspending the cells into a homogenous cell suspension;

a second in-line mixer in fluid connection with the first in-line mixer for mixing the homogenous cell suspension with a lysis solution to form a lysate; and

a third in-line mixer in fluid connection with the second in-line mixer for mixing the lysate with a salt solution to form a precipitate and a supernatant.

50. A method of purifying plasmid DNA from a cell, the method comprising the steps of:

lysing the cell with a lysis solution to form a lysate solution;

removing contaminants from the lysate solution by precipitating the contaminants from a supernatant of the lysate solution;

recovering plasmid DNA from the supernatant using a hydrophobic interaction column.

51. The method in claim 50 wherein the hydrophobic interaction column comprises a resin selected from a group consisting of: Octyl Sepharose 4 Fast Flow, Phenyl Sepharose, Butyl Sepharose, Phenyl 650-S.

52. The method in claim 50 wherein the step of recovering the plasmid DNA from the supernatant comprises the steps of:

adjusting a salt concentration of the supernatant to have at least 2M ammonium sulfate;

flowing the adjusted supernatant through the hydrophobic interaction column; and

eluting the plasmid DNA with an eluent solution having less than 2M ammonium sulfate.

53. The method in claim 52 wherein the eluent solution has ammonium sulfate at a concentration of less than or equal to 1.6M.

54. The method in claim 50 wherein the step of recovering plasmid DNA from the supernatant comprises the steps of:

adjusting a salt concentration of the supernatant to have about 1.6M to about 1.75M ammonium sulfate;

flowing the adjusted supernatant through the hydrophobic interacting column; and

collecting a flow through eluate flowing from the hydrophobic interacting column.

55. The method in claim 50 wherein the step of recovering plasmid DNA from the supernatant further involves the use of an anionic exchange column.

56. The method in claim 55 wherein the use of an anionic exchange column comprises the step of eluting plasmid DNA via a step gradient.

57. The method in claim 55 wherein the anionic exchange column comprises a resin having a particle size of 20-40 microns.

58. The method in claim 55 wherein the anionic exchange column comprises a resin selected from a group consisting of: Fractogel EMD TMAE (650-S), Fractogel (R) EMD TMAE Hicap, Q Sepharose 4 Fast Flow, DEAE 650-S

59. The method in claim 55 wherein the plasmid DNA is eluted from the anionic exchange column with a salt solution selected from a group consisting of: about 1.9 M ammonium sulfate and at least 0.7M NaCl.

60. The method in claim 50 wherein the step of recovering plasmid DNA from the supernatant comprises the steps of:

passing the supernatant through an anionic exchange column; wherein the plasmid DNA binds to the anionic exchange column;

eluting the plasmid DNA in a first eluate from the anionic exchange column;

adjusting a salt concentration of the first eluate to contain at least 2M ammonium sulfate;

passing the first eluate through the hydrophobic interaction column; wherein supercoiled plasmid DNA binds to the hydrophobic interaction column; and

eluting the supercoiled plasmid in a second eluate from the hydrophobic interaction column via an eluent having less than 2M ammonium sulfate.

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